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Some Parasites of Plaice *Pleuronectes platessa* L. in Three Different Farm Environments

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Introduction

Disease and parasite problems associated with the holding of animals in artificial and often crowded conditions are well documented in terrestrial farming and freshwater fish cultivation. However, because marine fish cultivation is a recent development, little information is available on applied aspects of parasitology in this field. Furthermore, there is a lack of basic information on parasites of juvenile marine fish generally. In an attempt to fill some of the gaps in our knowledge we undertook a sampling programme on two White Fish Authority experimental marine fish cultivation units: Hunterston in the Clyde estuary, where fish are reared in the warm water discharge from a nuclear power station, and Ardtoe off the west coast of Scotland, where they are reared under more natural conditions in sheltered inlets. The principal objectives of this study were as follows:—

1. To compare the faunas of hatchery-reared plaice under different farm conditions.

2. To compare the parasite faunas of hatchery-reared plaice with that of a wild population.

3. To examine the effect on the parasite fauna of transfer of plaice between different farm environments. Preliminary sampling at Hunterston indicated that, compared with wild populations, the plaice there had a sparse parasite fauna. As the Hunterston unit was intended partly as a hatchery supplying young stock to farms in more natural conditions, it was important to examine the effect on fish with little or no previous experience of parasites, and consequently little acquired resistance to infestation, of transfer into an environment where they would be exposed to a wide variety of parasite species.

4. To determine which parasites may be pathogenic to plaice and other flatfish in farm environments.

Material and Methods

From September 1970 to November 1971, 33 samples, totalling 326 0+ and 1+ plaice, were collected from three sites and examined for parasites. The sites (Fig. 1) were chosen for White Fish Authority project C. 70P.70.2 as being representative of different potential methods of commercial fish culture; our sampling programme was accommodated within this project.

All plaice were reared from the egg in the warm chlorinated sea water discharge from the Hunterston nuclear power station. The plaice population was first sampled at Hunterston, after which, in October 1970, two groups, each consisting of 360 plaice, were transferred to floating cages at two sea sites, an intertidal pond with limited water exchange (Ardtoe Pond) and a sheltered coastal site in Loch Moidart which presented a more natural environment (Moidart I). A further group of 360 plaice, having overwintered at Hunterston, were transferred to another cage at the Moidart site in April 1971 (Moidart II). Sampling of the two residual Hunterston

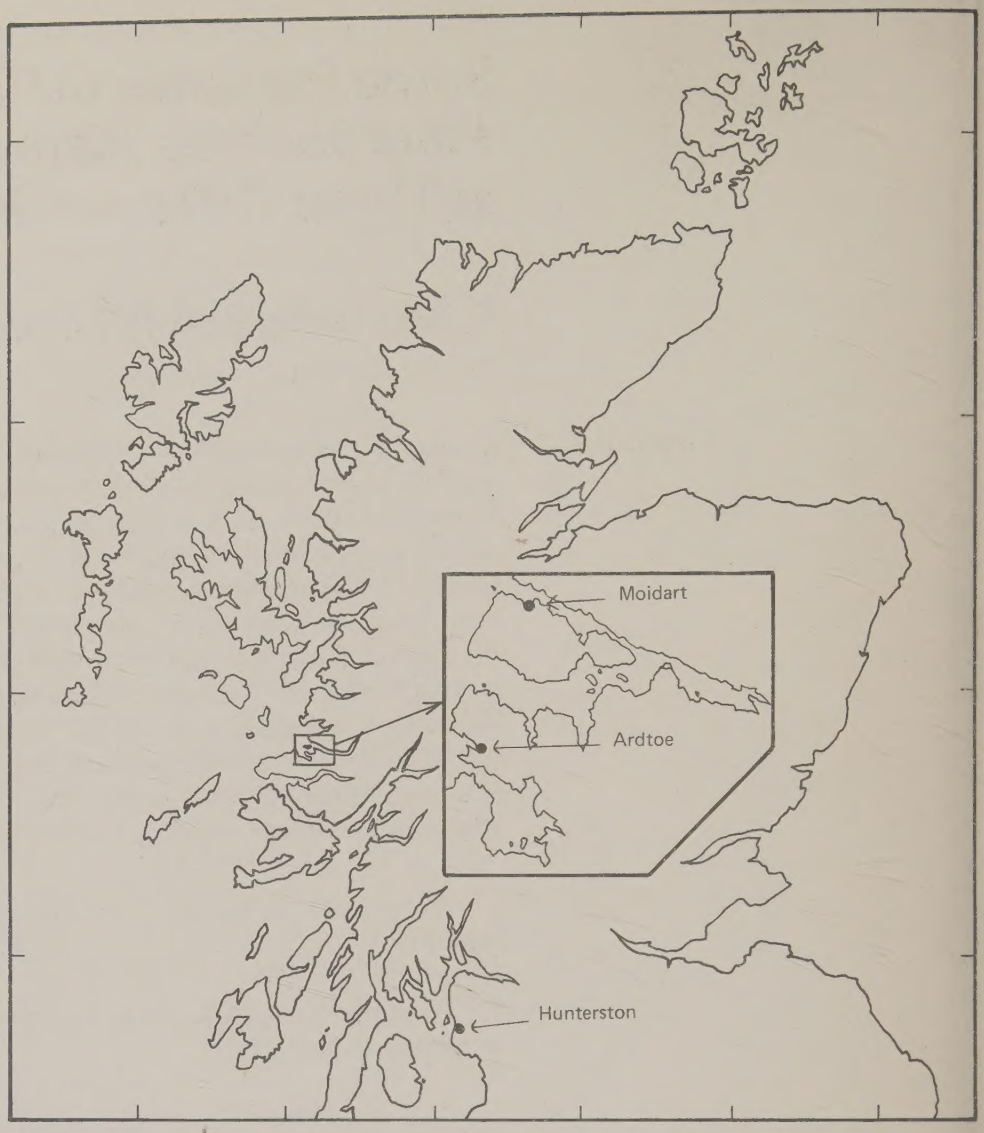

Figure 1. Map showing locations of the three farm environments studied.

populations, ie those remaining after the autumn and spring transfers (HI and HII respectively), was continued to the end of the experiment. Population densities were initially the same in all cages.

Sampling intervals varied from one month at the beginning of the experiment to three to six months in the later stages. Samples, normally of ten plaice each, were taken at random, killed and immediately preserved in 10% formol saline. Complete internal and external autopsies for protozoan and metazoan parasites were carried out in the laboratory. Slides were prepared by crushing or scraping tissues rather than by taking surface smears. A scraping of the entire organ was made in the case of the gills, gall and urinary bladders. Phase contrast microscopy was routinely used for searching such preparations and for identification of parasites, and an arbitrary scale was decided upon for measuring intensity of infestation of protozoan species. In metazoan infestations total numbers of parasites were counted. The figures quoted for a wild population are drawn from investigations conducted by MacKenzie (1968, 1971) at the Marine Laboratory's field station at Loch Ewe on the west coast of Scotland.

Results

Parasites with complex life cycles, particularly the Digenea and Cestoda, were not as common in farm stock as in the wild plaice population (Table I). The only digenean species common to and frequently occurring in all areas was the encysted metacercarial stage of *Cryptocotyle lingua*.

Table I

Comparison of the parasite faunas of wild and farmed plaice *Pleuronectes platessa*. Number of species in each parasite group

Parasite	Wild plaice*	Hunterston I & II	Ardtoe Pond	Moidart I & II
Protozoa	3	4	3	4
Monogenea	1	0	1	1
Digenea	10	3	1	2
Cestoda	4	0	0	0
Nematoda	2	0	0	2
Acanthocephala	1	0	0	0
Crustacea	1	0	0	1

*Data from MacKenzie (1968, 1971).

Protozoa

Glugea stephani (Hagenmüller, 1899). This intracellular microsporidian was found encysted in the walls of the intestine and rectum and occasionally in the mesenteries. At Hunterston the incidence of infestation was 6–8% and only one lightly infested plaice was found elsewhere (Table II). No heavy infestations, as defined by McVicar (1975), were found in the present study.

Unidentified microsporidians. Two fish from Hunterston II were infested with unidentified microsporidian parasites. In one, cysts containing spores 4.4 x 2.5μ were found throughout the soft tissues; in the other, a single cyst containing spores 5.1 x 2.7μ was found in a primary gill lamella.

Sphaerospora irregularis Kabata, 1962. This myxosporidian occurred commonly in the urinary bladder, particularly at Ardtoe Pond and Moidart I (Table II). Infestations were usually light (estimated 10–190 spores per smear) to medium (200–990 spores per smear) (Table III). At Ardtoe Pond and Moidart I, incidence of infestation increased rapidly over the first two months to a peak of 90%, then gradually decreased over the following five months to disappear by August 1971 (Fig. 2). At Hunterston, an early 50% incidence of infestation disappeared within three months, but part of the same stock later transferred to Moidart II contracted a low level of infestation recorded in only one sample.

Scyphidia adunconucleata MacKenzie, 1969. Only six fish were infested with this ciliate on the skin and gill surfaces, four of them from the first sample taken at Hunterston in September 1970. All infestations were light (Tables II and III).

Trichodina borealis (Dogiel, 1940). This ciliate occurred on the gills of plaice from all sites with no clear pattern of infestation emerging (Fig. 3). The most spectacular variations occurred at Hunterston II, where incidence increased from zero to 100% within three months and then decreased to 30% one month later. Estimated intensities of infestation ranged from 10 to 3000 per fish.

Table II

Incidence of parasite infestation (%) in farmed plaice. All samples

	Hunterston		Ardtoe pond	Moidart	
	I	II		I	II
Number of fish examined	90	50	68	70	48
Protozoa					
Glugea stephani	8	6	0	0	2
unidentified microsporidians	0	4	0	0	0
Sphaerospora irregularis	8	0	44	43	6
Scyphidia adunconucleata	4	2	2	0	0
Trichodina borealis	3	26	9	20	27
Monogenea					
Gyrodactylus unicopula	0	0	7	30	17
Digenea					
Cryptocotyle lingua	78	82	91	94	65
Hemiurus communis	4	8	0	1	0
Lecithaster gibbosus	1	0	0	0	0
Nematoda					
Contracaecum sp.	0	0	0	1	2
Anisakis sp. larva	0	0	0	1	0
Crustacea					
'Chalimus' larva	0	0	0	1	0

KEY

H I:— Hunterston I
Pond:— Ardtoe Pond
M I:— Moidart I
H II:— Hunterston II
M II:— Moidart II

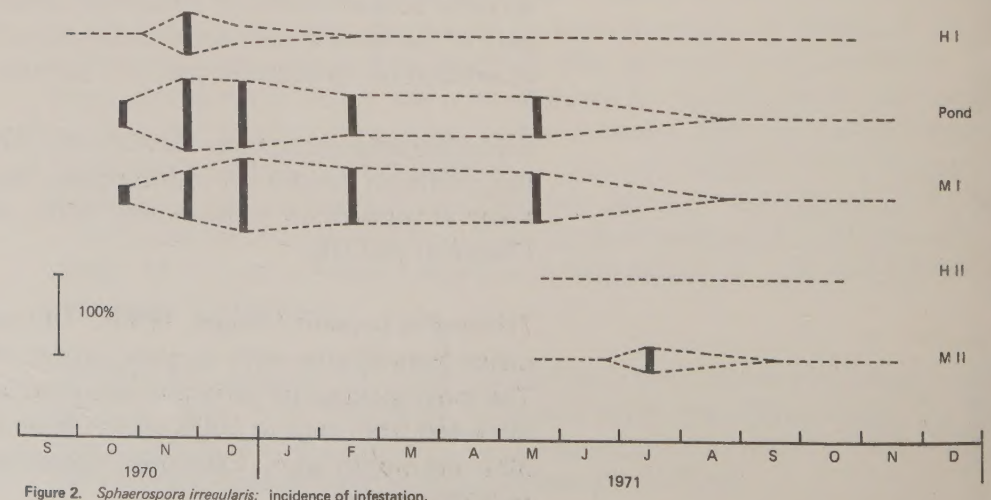

Figure 2. *Sphaerospora irregularis:* incidence of infestation.

Table III

Intensity of infestation, as mean level per infested fish, in farmed plaice. All samples

L = light, M = medium infestation (see text)

	Hunterston		Ardtoe pond	Moidart	
	I	II		I	II
Number of fish examined	90	50	68	70	48
Protozoa					
Glugea stephani	M	M	0	0	L
Sphaerospora irregularis	L	0	L	M	M
Scyphidia adunconucleata	M	L	L	0	0
Trichodina borealis	M	L	L	L	M
Monogenea					
Gyrodactylus unicopula	0	0	23.0	93.3	24.4
Digenea					
Cryptocotyle lingua	3.5	5.0	6.7	6.2	5.9
Hemiurus communis	1.0	1.3	0	1.0	0
Lecithaster gibbosus	1.0	0	0	0	0
Nematoda					
Contracaecum sp.	0	0	0	1.0	2.0
Anisakis sp. larva	0	0	0	1.0	0
Crustacea					
'Chalimus' larva	0	0	0	1.0	0

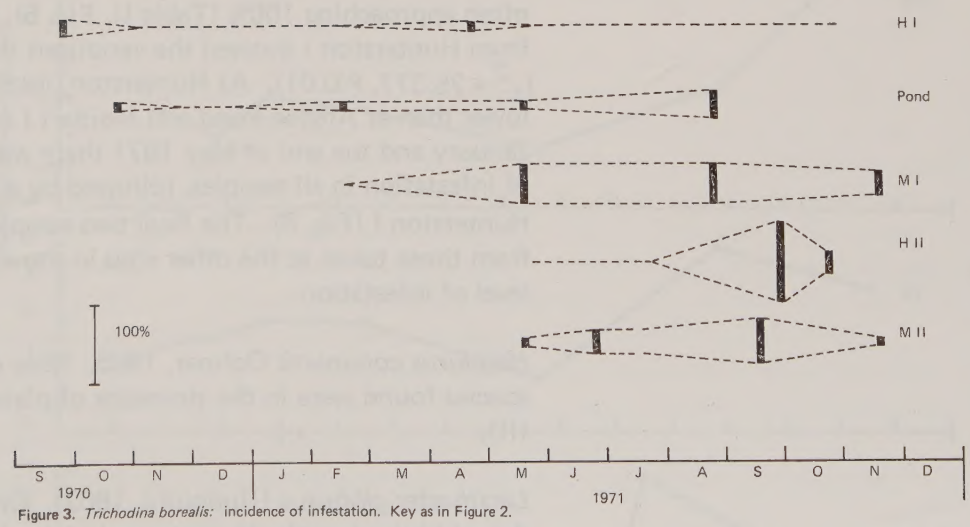

Figure 3. *Trichodina borealis:* incidence of infestation. Key as in Figure 2.

Monogenea

Gyrodactylus unicopula Glukhova, 1955. An important feature of this study was the absence of this parasite from all Hunterston samples, although it occurred at Ardtoe Pond and Moidart I and II (Tables II and III). It can therefore be stated with certainty that these infestations were acquired after transfer from Hunterston. In this study *G. unicopula* was found on the gill filaments only, but it is also known to occur on the skin and fin surfaces of plaice (MacKenzie, 1970, and unpublished results). Intensity of infestation ranged from one to 618 parasites per fish. At Moidart I, incidence of infestation increased from 10% in February 1971 to 100% in August 1971 with a corresponding increase in intensity of infestation to the highest recorded mean intensity of 174.4 per fish (Fig. 4). Three months later the level of infestation had decreased considerably without treatment of any kind. A natural decrease of 50% incidence to zero was also observed in Ardtoe Pond.

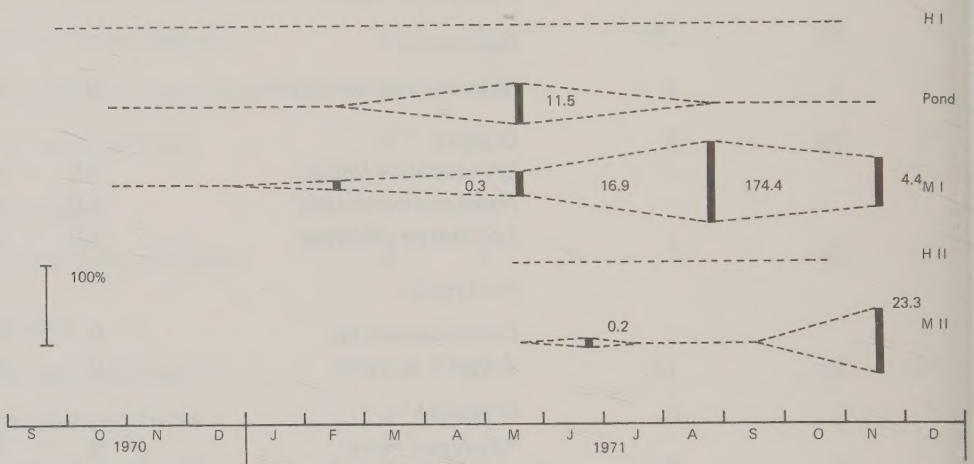

Figure 4. *Gyrodactylus unicopula:* incidence of infestation. Figures are intensity of infestation as mean number of parasites per fish. Key as in Figure 2.

Digenea

Cryptocotyle lingua (Creplin, 1825). Metacercariae of this species were found encysted in the skin, fins, gills and musculature of plaice from all samples. They were frequently found at the bases of stunted and bifurcated gill lamellae, particularly in the earlier samples. In later samples the number of cysts in the gill lamellae had increased, but some deformed gill lamellae were found without associated cysts. Incidences in samples were generally high, often approaching 100% (Table II, Fig. 5), but a test of homogeneity on those from Hunterston I showed the variations there to be highly significant ($\chi^2 = 25.377$, $P<0.01$). At Hunterston, intensity of infestation was generally lower than at Ardtoe Pond and Moidart I and II (Table III). Between January and the end of May 1971 there was an overall decrease in intensity of infestation in all samples, followed by a marked increase at all areas except Hunterston I (Fig. 6). The final two samples taken at Moidart II differed from those taken at the other sites in showing a significant decrease in the level of infestation.

Hemiurus communis Odhner, 1905. Nine of the ten specimens of this species found were in the stomachs of plaice from Hunterston (Tables II and III).

Lecithaster gibbosus (Rudolphi, 1802). Only one specimen was found, in the mid-intestine of a Hunterston I plaice (Table II).

Nematoda

Two *Contracaecum* sp. larvae and one adult *Contracaecum aduncum* (Rudolphi, 1802) were found in the stomachs of two plaice from Moidart I and II, and one *Anisakis* sp. larva from the fore-intestine of a Moidart I fish.

Crustacea

A single "chalimus" larva, ie an early stage of a caligid copepod, was noted attached to the upper surface of the tail fin of a Moidart I plaice.

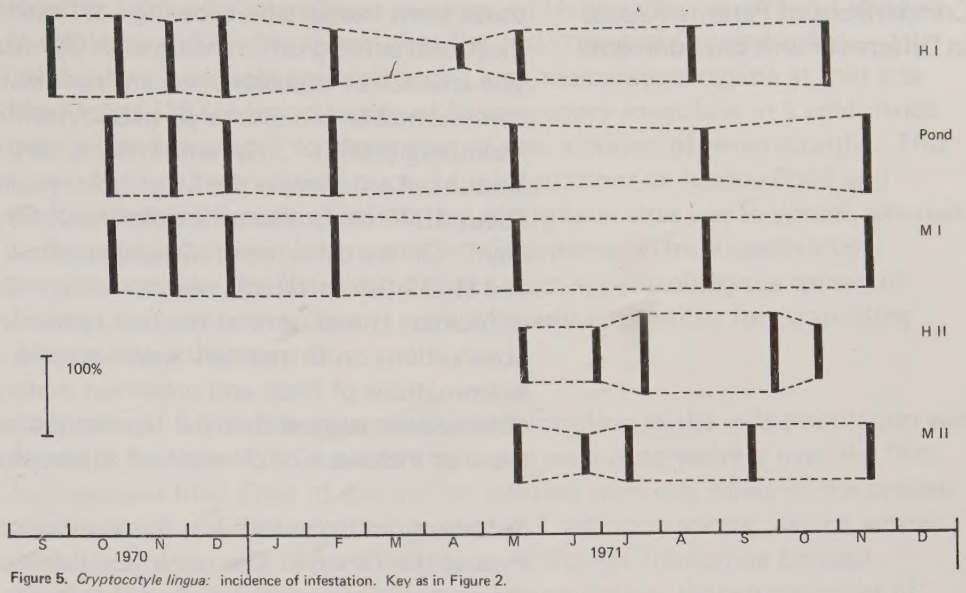

Figure 5. *Cryptocotyle lingua:* incidence of infestation. Key as in Figure 2.

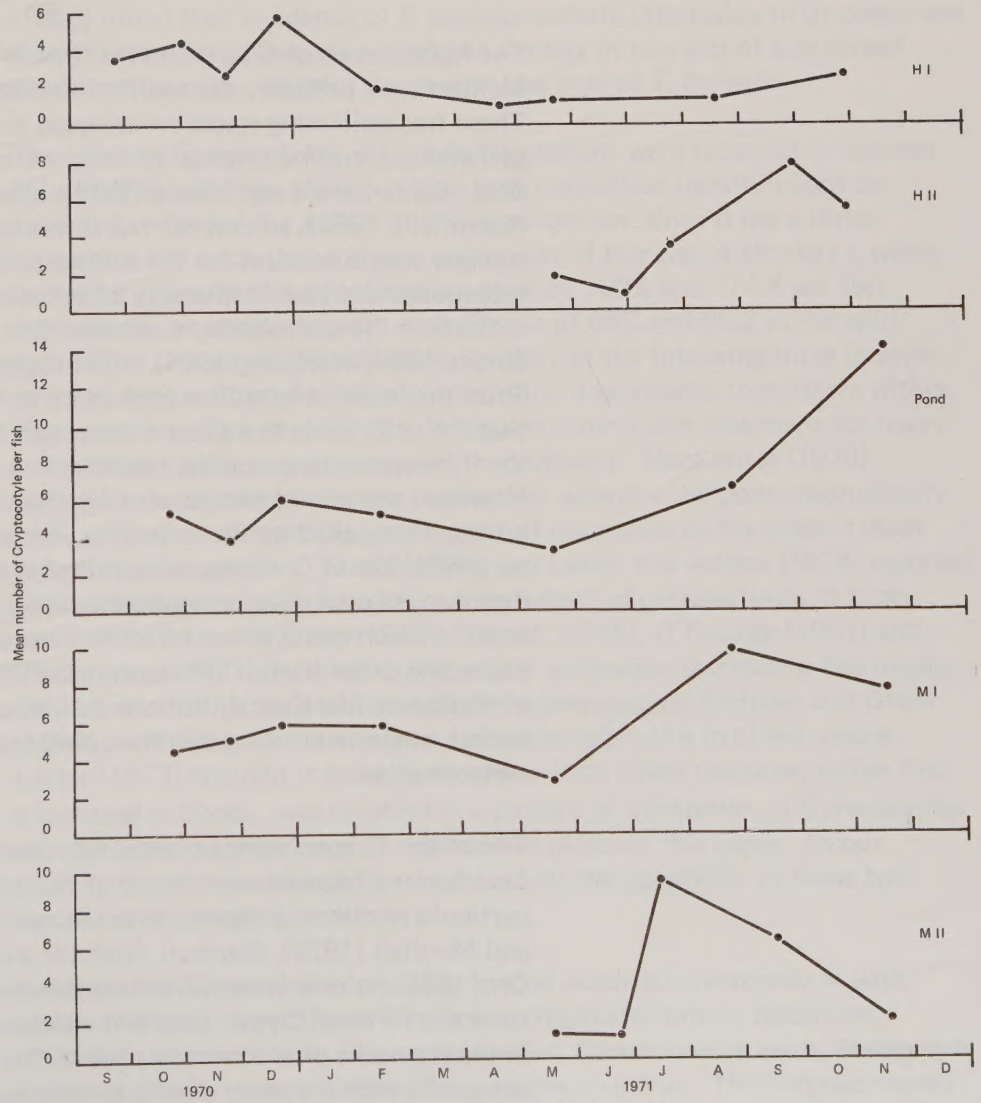

Figure 6. *Cryptocotyle lingua:* intensity of infestation as mean number of parasites per fish. Key as in Figure 2.

Discussion

Comparisons of Parasite Faunas in Different Farm Environments

The parasite fauna of these plaice generally formed two distinct groups, those from Hunterston showing a different pattern from the others. The most outstanding difference was in *Gyrodactylus unicopula* infestations. The absence of this monogenean from Hunterston may be attributable to chlorination of the discharge water from the power station. According to Malmberg (1966) "...*Gyrodactylus* is very sensitive to chlorine. The small amount of chlorine in the Stockholm municipal water at a temperature of about 10°C destroyed all specimens of *Gyrodactylus* species within one day." On the other hand, *Glugea stephani* occurred at both Hunterston I and II (11 fish infested), whereas only one lightly infested plaice was found at Moidart II and none at the two remaining sites. Other unpublished observations on *G. stephani* have revealed a discrepancy between results from examinations of fresh and preserved material from the same source. These observations suggest that the figures quoted in this study may underestimate the true incidence of *G. stephani* infestation.

Sphaerospora irregularis was much more common at Ardtoe Pond and Moidart than at Hunterston. The minimum duration of an infestation, as measured by the presence of spores, was found to be about seven months at Ardtoe Pond and Moidart I, but only one month at Hunterston I. Using this criterion we may have measured the duration either of the infestation itself or of the period of spore formation, since other stages of the parasite may have remained undetected in the walls of the urinary bladder. The factors responsible for the restriction of the infestation at Hunterston are unknown.

The higher levels of *Cryptocotyle lingua* infestation at Ardtoe Pond and Moidart were probably the result of exposure to greater numbers of cercariae. These free-swimming stages are released in very large numbers from the periwinkle *Littorina littorea,* which occurs commonly on the rocks at these sites. Cercariae are not released below about 10°C (Sindermann and Rosenfield, 1954), so that the rise in sea temperature to above that level in summer would account for the sudden increase in infestation observed. The subsequent decrease in intensity of infestation between January and May 197 indicates a life span shorter than reports in the literature (Hoffmann, 1967; Shigin, 1964; Wolfgang, 1954) might suggest for metacercariae in fish hosts. Since the initial infestations took place at Hunterston, it was considered that the chlorine in the water there might have had some adverse effect on the free-swimming cercariae, resulting in a shortened metacercarial life span. However, this would not account for the corresponding pattern observed in infestations picked up the following year at Moidart. As far as we are aware, no similar loss of *C. lingua* infestation has been previously reported. This digenean is best known as a parasite of fish, particularly gadoids, found in rocky environments where *Littorina littorea* is common, and it may be that plaice and other flatfish are less suitable hosts. An experiment similar to that of McQueen, MacKenzie, Roberts and Young (1973), but over an extended period, might help to explain the unexpectedly short life span of these metacercariae.

Ten of the 11 specimens of hemiurid digeneans *(Hemiurus communis* and *Lecithaster gibbosus)* were found at Hunterston. Planktonic invertebrates, particularly calanoid copepods, act as second intermediate hosts of this group, and Marshall (1925), Marshall, Nicholls and Orr (1934), and Marshall and Orr (1955) reported hemiurid metacercariae in *Acartia clausi* and *Calanus* sp. from the Firth of Clyde. Calanoid copepod remains were occasionally found in the stomachs of Hunterston plaice. The higher infestation at Hunterston compared with the other sites may have been due to differences between these areas in the abundance of the parasites or to planktonic invertebrates forming a greater part of the diet of Hunterston plaice.

Comparison of Parasite Faunas of Wild and Farm Plaice

All three protozoan species found in wild plaice were present in farm stock, together with a fourth, *Glugea stephani,* at Hunterston I and II and Moidart II. McVicar (1975) has shown that the occurrence of *G. stephani* at Hunterston is probably related to the high temperature regime at that site. MacKenzie (1971) found spores of *Sphaerospora irregularis* in a wild plaice population from April to November, ie over a period of seven months. This is also the minimum period quoted for infestations at Ardtoe Pond and Moidart I, but the peak of infestation at the farm sites was in winter, whereas in the wild population it occurred in mid-summer. This suggests that temperature may not be an important factor in controlling the spread of *S. irregularis,* and other factors were probably responsible for controlling infestations at Hunterston.

Incidence of *Scyphidia adunconucleata* infestation in the wild population was found to be highest in 0+ plaice and to decrease progressively over the first two years of life. Four of the six fish infested with this ciliate in the present study were 0+. These results suggest that *S. adunconucleata* may be almost exclusively a parasite of juvenile plaice. Although *Trichodina borealis* occurred throughout the year in the wild population, there were peaks of infestation in September and October in the 0+ and 1+ age groups. Maximum infestations at Hunterston II and Moidart II also occurred in September, suggesting a seasonal cycle of development. Infestations with both ciliate species were generally lighter in farm than in wild plaice, which is surprising in view of the greater population densities in the farm stock. MacKenzie (1968) noted that incidence of *S. adunconucleata* infestation in 0+ plaice was greater at the lower level of population density in two sets of submerged experimental cages, whereas the reverse was true of *T. borealis.*

The heaviest *Gyrodactylus unicopula* infestations were recorded in summer in both wild and farm plaice. A high host population density might be expected to favour the spread of this monogenean, since it has a direct single-host life cycle, but the only indication of this was at Moidart I, where incidence and intensity of infestation reached 100% and 174.4 per fish respectively, as compared with peak figures of 68% and 66.2 in the wild population. However, this decreased rapidly in the following three months, while at Ardtoe Pond a moderate infestation disappeared completely within three months. This suggests that in these environments treatment for heavy infestations of this parasite may not be necessary. MacKenzie (1970) suggested the existence of some mechanism whereby the population density of *G. unicopula* is normally controlled and the results of the present study support this suggestion. Khalil (1964) and Lester and Adams (1974) reported similar phenomena with laboratory infestations of gyrodactylids in fresh-water fish. Jahn and Kuhn (1932), Nigrelli (1935), O'Rourke (1961) and Vladimirov (1971) suggested that humoral antibodies secreted in fish mucus may be responsible for resistance to monogeneans, and Fletcher and Grant (1969) demonstrated the presence of immunoglobulins in plaice mucus. Lester (1972) thought it more likely that a local tissue response, rather than a humoral antibody, was involved in a pattern of infestation of *Gyrodactylus* on sticklebacks similar to that reported on plaice in this paper. In our opinion current knowledge does not exclude the possibility of these two mechanisms operating together.

Amongst the Digenea, the following species occurred commonly in wild plaice, but were absent from farm stock: *Stephanostomum baccatum, Rhipidocotyle minimum, Derogenes varicus, Podocotyle atomon, Podocotyle staffordi, Plagioporus varius* and *Zoogonoides viviparus.* The first two named occur as metacercariae in the musculature and other soft tissues, and the remainder as adult worms in the alimentary tract. This experiment has demonstrated

that *S. baccatum* and *R. minimum* can be effectively controlled by raising the cages above the sea bed, since MacKenzie (1968) found both species occurring commonly in plaice held in submerged cages. Wolfgang (1955) stated that the cercaria of *S. baccatum* tends to crawl or lie on the substratum and none was seen to rise more than a few millimetres from the bottom. The incidental control of this parasite is particularly rewarding, as the presence of large numbers of cysts in the flesh could adversely affect the market value of farm fish. The cercaria of *R. minimum* is unknown, but these results suggest that it also is adapted to a benthic habitat. The absence of the six adult digeneans named above may be attributed to the absence of their invertebrate second intermediate hosts from the diet of farm plaice.

Cryptocotyle lingua metacercariae were more common in farm than in wild plaice. MacKenzie (1968) similarly found that this parasite occurred more frequently in plaice held in submerged cages than in the wild population. Wild juvenile plaice occur only in sandy environments, whereas the periwinkle, the first intermediate host of *C. lingua,* is found on rocks. The submerged cages at Loch Ewe and the floating cages at Moidart and Ardtoe were set in habitats closer to rocks carrying periwinkle populations than those in which plaice would occur naturally. The shore line at Hunterston close to the source of the farm's water supply is also rocky. The farm plaice were all therefore probably exposed to larger numbers of *C. lingua* cercariae than most wild populations. Another important factor may be that under natural conditions plaice spend a considerable amount of their time buried in sand and are thereby protected from infestation with *C. lingua,* whereas in cages or tanks they are denied this protection.

Hemiurus communis was not a common parasite of the wild population, probably because calanoid copepods are not normally a major item in the diet of juvenile plaice (Edwards and Steele, 1968). The incidence of *H. communis* infestation at Hunterston was similar to that in the wild population, and it was noted that calanoid copepod remains occurred more frequently in the stomach contents of Hunterston plaice than in those from Ardtoe and Moidart.

Of the remaining parasites, only the nematode *Contracaecum aduncum* is of any obvious significance in the present context, since the others occurred only rarely in wild and farm plaice. In the wild population, *C. aduncum* were present mainly as larvae in the visceral cavity and none was found in the stomach, whereas in farm plaice all three specimens were in the stomach. These were probably ingested with the raw minced fish and shellfish diet which may well have contained this ubiquitous nematode.

Effect of Transfer of Hosts on Parasite Fauna

Compared with wild plaice populations, fish from the cages at Ardtoe and Moidart carried a small number of parasite species. It is not possible therefore within the context of this paper to examine fully the implications of transferring plaice reared in an environment containing few parasites to one in which they are exposed to a wide range of their natural parasites. Under different cultural conditions the parasite fauna and its effect on the fish may be entirely different. The monogenean *Gyrodactylus unicopula* provides one example of the type of host-parasite interaction which may occur in these situations, as it was established earlier that these plaice could not have encountered this parasite prior to their transfer from Hunterston. The length of time between transfer and initial infestation and the further long period before heavy infestations occurred indicate that under these conditions infestation with *G. unicopula* does not proceed rapidly and uncontrollably.

The absence of *Glugea stephani* from wild plaice in Scottish waters indicated that the parasite was probably transfered into Hunterston with stock from more southern waters (McVicar, 1975). The single record of the parasite at Moidart II probably arose from transfer of a fish infested at Hunterston, as we believe that Scottish coastal temperatures are too low for successful transmission of this parasite. Although no serious consequences arose from its transfer to Moidart, this instance illustrates a further risk involved in transferring fish from one locality to another.

Potential Pathogens

Although they presented no problems in the present investigation, we have evidence from our general experience with plaice in captivity that certain parasites with direct life cycles could prove troublesome. Concurrent infestations of *Trichodina borealis* and *Gyrodactylus unicopula* have had more serious pathological effects than either species alone, with massive skin lesions forming and heavy mortalities of untreated fish occurring. Similarly, Pearse (1972) recorded deaths in hatchery reared plaice in the Isle of Man only during mixed outbreaks of the same two parasites. Noble (1963) found a statistically positive association between numbers of *Trichodina* and *Gyrodactylus* on the gills of *Gillichthys mirabilis* and speculated that the *Trichodina* fed on host tissues damaged by the monogenean and other metazoan parasites.

Phelps (1971) reported a fish kill in Kentucky associated with heavy infestations of *Trichodina* and other ciliates, and Lom (1969, 1973) noted that whenever adverse conditions prevailed against the fish, including concomitant or earlier invasion by other parasites, trichodinids may proliferate, causing severe lesions if they penetrate the skin surface. We achieved effective control in plaice by repeated fresh water dips and sprays. McVicar (1975) has also shown that *Glugea stephani* caused plaice mortalities under both experimental and farm conditions.

In the present investigation parasites with life cycles involving more than one host appeared to be of little or no importance as pathogens. This may be attributed to the scarcity of suitable intermediate or definitive hosts, making completion of the life cycle difficult or impossible. Where plaice are reared in different cultural conditions, eg together with cultured bivalve molluscs which may harbour gasterostome digeneans, the opportunities for these parasites may be greater, and consequently those which include plaice in their range of intermediate hosts may assume greater importance. MacKenzie (1971) and MacKenzie and Liversidge (1975) have demonstrated that massive infestations of metacercariae of *Cryptocotyle lingua* and *Stephanostomum baccatum* can cause mortalities of O-group plaice. The nematode *Contracaecum aduncum* may also come into this category (Steele, 1966; MacKenzie, 1968). The parasite groups which include the species referred to above occur widely in marine fish generally, and particularly in other flatfish, some of which may become of considerable importance to fish farming. They should therefore be taken seriously whenever they occur in farm stock. At the same time, it must be emphasized that good husbandry techniques and some knowledge of the biology of the most important pathogens should provide a basis for satisfactory control of most of these parasites.

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Summary Over a period of fifteen months, young plaice *(Pleuronectes platessa* L.) wer held in three different experimental farm environments and periodically examined for parasites. These environments were: onshore tanks with heated chlorinated water (Hunterston); floating cages in a marine pond (Ardtoe); and floating cages in a sea loch (Loch Moidart). The parasite faun of the Hunterston farm fish showed a different pattern from the others, notably in the complete absence of *Gyrodactylus unicopula.* This was in contrast to the heavy infestations in August in Loch Moidart which, by November, had decreased considerably without treatment despite a history of no previous infestation. Several digenean metacercariae which are comm in wild plaice were absent from farm fish, although one, *Cryptocotyle lingua* was more common in farm stock than in wild fish. The transfer of *Glugea stephani* from Hunterston to Moidart indicates the need for caution with movements of farm stock. Concurrent infestations of *Trichodina borealis* a *Gyrodactylus unicopula* appear to be more detrimental to farm plaice stock than either species alone. Rearing benthic flatfish in floating cages avoids some common infestations involving parasites with benthic intermediate stages.

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